PATENT APPLICATION

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re the Application of

Didier RAOULT et al. Group Art Unit: 1645

Application No.: 09/936,921 Examiner: P. Baskar

Filed: September 24, 2001 Docket No.: 110530

For: DIAGNOSIS OF WHIPPLE'S DISEASE

DECLARATION UNDER 37 C.F.R. §1.132

I, M. Drancourt, a citizen of France, hereby declare and state:

a. I have a Ph. D. in Cell Biology and microbiology , which was conferred upon me by Université Aix-Marseille II in Marseille, France in 1988.

b. I have been employed by Université de la Méditérannée since 1990 and I have had a total of 20 years of work and research experience in Medical Microbiology.

c. I am a member of Société Française de Microbiologie.

d. My publications include the following works in this field: Drancourt, "*Tropheryma whippelii,* pathogène émergent à culture intracellulaire responsable de la maladie de Whipple". La Presse Médicale (1999; 28:435-439) (hereinafter "my 1999 Presse Medicale article"); Drancourt M, Raoult D, Lepidi H, Fenollar F, Birg ML, Bodaghi B, Hoang PL, Lelievre JD. Culture of *Tropheryma whippelii* from the vitreous fluid of a patient presenting with unilateral uveitis. Ann Intern Med. (2003; 139:1046-7); Raoult D, Ogata H, Audic S, Robert C, Suhre K, Drancourt M, Claverie JM. *Tropheryma whipplei* Twist: a human pathogenic Actinobacteria with a reduced genome. Genome Res. (2003; 13:1800-9); and Drancourt M, Carlioz A, Raoult D. rpoB sequence analysis of cultured *Tropheryma whippelii*. J Clin Microbiol. (2001; 39:2425-30).

e. I have reviewed the following article: Schoedon et al., "Deactivation of macrophages with interleukin-4 is the key to the isolation of *Tropheryma whippelii*". Journal of Infectious Diseases (1997; 176:672-677) (hereinafter "Schoedon"). In particular, I studied this article in connection with the preparation of my 1999 Presse Medicale article, which is identified above. In addition, I have subsequently reviewed this article in the preparation of this Declaration.

f. I have further reviewed about all the State of the Art in the field of *Tropheryma whipplei* cultivation, including the publications by Pr Raoult's team and the patent application WO0058440 corresponding to the above-identified U.S. patent application, and inter alia the following scientific publications in the preparation of this Declaration:

- Hinrikson et al. "Detection of three different types of Tropheryma whippelii" in International Journal of Systematic Bacteriology (1999; 49:1701-1706) (hereinafter "Hinrikson"),

- the abstract by Muller et al. "Cultivation of T. whippelii" in Gastroenterology vol.116, n°4, part 2, April 1999, page A910, Abstract XP002123745 (hereinafter "Muller"),

- Maiwald et al. "Cultivation of Tropheryma Whipplei from Cerebrospinal Fluid" Journal of Infectious Disease (2003; 188:801-808) (hereinafter "Maiwald"), and

- Bentley et al. "Sequencing and analysis of the genome of the Whipple's disease bacterium Tropheryma whipplei" The Lancet (2003; 361:637-644) (hereinafter "Bentley").

g. I have further reviewed in the preparation of this Declaration:

- the attached letter by Schoedon dated 22 December 1998 sent to myself, and

- the laboratory books of Professor Raoult's team concerning culture of the *Tropheryma whipplei*.

I can now declare and state:

1. Schoedon and Muller report studies carried out on cultures of bacteria they reported as *Tropheryma whipplei* in human blood monocytes. They claimed to have isolated 2 such isolates. Such a culture was abandoned as recognized by Pr Schoedon in the above mentioned letter. Also, none of the 2 isolates has been made available in a strain deposit collection contrary to international code in microbiology, which asks that new isolates be deposited in public collection while it has long been sought to obtain such culture by the scientists in this area.

2. Schoedon and Muller have never more published since these first articles in 1997 and 1999 on that subject while their findings was of very great importance and had been long sought in this area. (see Maiwald, page 801: "*Cultivation of this bacterium has therefore been a goal of clinicians and microbiologists for several decades*".)

3. I can testify that Pr Raoult's team has attempted to reproduce the results reported by Schoedon and Muller in following their experimental teaching and these attempts were unsuccessful.

4. Other teams involved in this technical field have also unsuccessfully attempted to confirm the results of Schoedon. In particular, as described in Maiwald, the finding by Schoedon of the propagation of bacteria could not be confirmed in subsequent studies. Maiwald, p. 802, col. 1, lines 4-9: "*..investigators inoculated interleukin-4-deactivated macrophages with heart-valve tissue affected by WD and reported propagations [8]. However, this finding could not be confirmed in subsequent studies [9]*" (Zaaijer et al.), and p. 805, col. 2, lines 2-5: "*A previous report [8], describing the growth of T. whipplei in interleukin-4-deactivated macrophages, has not been confirmed, either by us (M.M. and D.A.R., unpublished results) or by other investigators [9]*". This was further confirmed by two of the authors of Schoedon,

Martin Altwegg and Fabrizio Dutly, who indicated in an article published about two years after Schoedon (Hinrikson), that the relationship between clinical manifestations of Whipple's disease and different infecting strains of *Tropheryma whippeli* has not been studied "*mainly because of the absence of reliable cultures* (Schoedon *et al.*, 1997)." Hinrikson, p. 1705, col. 1, discussion. In fact, the summary of this article on page 1701 refers to *Tropheryma whippelii* as "*the uncultivated causative agent of Whipple's disease*" (emphasis added). In addition, the article indicates on page 1706 that certain studies are "*not feasible due to the fact that 'T. whippelii' has not yet been cultured on artificial media*". See also Raoult et al., The New England Journal of Medicine, Vol. 342, No. 9, pp. 620-625 (March 2, 2000), which indicates that the isolate described in Schoedon "*could not be subcultured*" (p. 620, col. 2).

5. Muller purports to describe the cultivation of *Tropheryma whippelii* in peripheral blood mononuclear cells (PMNC) treated with IL-4 and the cocultivation of *Tropheryma whippelii* in PMNC with macrophages and with the monocytic cell line U937, both deactivated by IL-4 pretreatment. The abstract indicates that its results "*were positive suggesting true bacterial growth in those cells*" and that treatment with IL-4 "*seems to induce replication of T. whippelii*" (emphasis added). Based on use of the terms "*suggesting*" and "*seems*," it is clear that the authors could not conclusively say that the bacteria had reproducibly multiplied in this culture. In addition, it is noted that this abstract has never been followed by a corresponding scientific publication.

6. By contrast the successful culture of *Tropheryma whipplei* according to the culture conditions taught by Raoult et al. have been confirmed by Pr Relman's team (see Maiwald and Bentley).

7. With the requirements in force today for the identification of bacteria by molecular biology, the results published by Schoedon would not be accepted as a definite

evidence that they have successfully cultivated the *Tropheryma whipplei* bacterium. Indeed, in their article the identification of the bacterium is based on a PCR with primers drawn from the 16S r DNA of the *Tropheryma whipplei* bacterium, which are universal primers and not considered as sufficiently specific of any bacterium. As acknowledged by Hinrikson, Dutly and Altwegg in their article of 1999 (Hinrikson, page 1701 column 2: "Several diagnostic *T. whippelii*-PCRs that target parts of the 16S rDNA have been established (Relman *et al.* 1992; . . Altwegg *et al.* 1996. . .). However, such systems may not discriminate between closely related species..."). Besides the PCR products obtained by Schoedon have not been sequenced, as now required for reliable identification of a new bacterium, but they have only be subjected to Southern blotting using an oligonucleotide of the 16S rRNA gene. The primers used by Schoedon were published by Relman (reference 4 of Schoedon), Maiwald (reference 13 of Schoedon) and Altwegg (reference 12 of Schoedon) and these authors have stated that they couldn't reproduce the experiments of Schoedon (see Maiwald and Hinrikson).

8. The kinetics results reported by Schoedon are not compatible with the more recent data concerning this bacterium. Indeed, reported kinetics of growth such as in Figure 4 and Figure 5 of Schoedon are absolutely not compatible with what was later known about the growth kinetics of *Tropheryma whipplei*. In the histogram of figure 4 as well as in the photographs of figure 5, they report passing from about 15-20% of cells infected by the bacteria to more than 50% in 48 hr. Such kinetics implies a doubling time of the bacteria lower than 24 hr. Such result is an extraordinarily rapid growth while *Tropheryma whipplei* is on the contrary particularly slow in growth with a doubling time of 18 days. At the time, it was not known that the doubling time of the *Tropheryma whipplei* bacterium was of 18 days.

9. It is likely that an organism mimicking some of the *Tropheryma whipplei* characteristics (intracellular growth, bacillary morphology, periodic acid-schiff positivity, reactivity with "TW-1/TW-3 or TW-1/TW-2" PCR primers) contaminated the Schoedon culture leading to the credence that *Tropheryma whipplei* had been isolated and cultured.

10. Another reason for the positive results reported by Schoedon could be that the initial inoculum they used was exceptionally high. This could be the possible reason why Schoedon was capable of detecting bacteria after 8-10 days of incubation followed by a passage and a further incubation of 10 days. However such exceptional circumstances, if any, in no way could prove that the *Tropheryma whipplei* bacterium was established in culture. Indeed, the man skilled in the art knows that to establish an intracellular bacterium in culture by using ex-vivo cells as did Schoedon, you can't obtain multiplication of the bacteria if the incubation period between two passages is lower than the doubling time of the bacterium. Under such conditions, you obtain a dilution of the bacterium as the ratio bacteria/cells numbers necessarily decreases and you will not be able to detect any bacterium after 3 or 4 passages, depending on the amount of bacteria in the initial inoculum. This could have been the reason why Schoedon did not report detection of bacteria after more than 4 passages under the conditions he taught, namely with passages after only 8-10 days of incubation and therefore was not able to depose the isolate in a bacterial collection and was led to "abandon the culture".For one of ordinary skill in the art, it is implicit in the phrases "established in culture" and "multiplies over time "that the ratio of bacteria to cells increases. In addition, it is also implicit in these phrases that the culture can be maintained over time , i.e. indefinitely.

11. In any event, it is practically and biologically impossible to establish in culture this *Tropheryma whipplei* bacterium for diagnostic purpose, in Il4 deactivated human monocytes as reported by Schoedon because the mean lifetime of monocytes is only 30 days, and human monocytes can't be furnished in sufficient amount to establish a bacterium in culture for biological diagnostic purpose. These cells are the more so unsuitable in view of the doubling time of the bacterium as explained above.

12. My 1999 Presse Medicale review article provided a summary of various articles concerning *Tropheryma whippelii*. It does not set forth the results of further experimentation. In particular, in my 1999 Presse Medicale article, I indicated that two strains of *Tropheryma whippelii* were isolated in a cell culture from two heart valves sampled from two different patients and that the strains were subsequently cultivated in a human line of monoblasts SigM5. In support of these statements, I referred to Schoedon. My statements do not reflect any experimentation that I conducted to confirm the accuracy or repeatability of the work described in Schoedon. Instead, this paper merely summarized the work of researchers in the field such as reported in the scientific literature, including Schoedon.

13. In view of all the above considerations, based on my own investigation and the more recent scientific publications by other investigators, it can be recognized that Raoult et al. (i.e., the inventors of the above-identified U.S. patent application) have been the first to teach the suitable conditions to establish the Tropheryma whipplei bacterium in culture as acknowledged in Maiwald (see the abstract: "*..many attempts have been made to cultivate this bacterium in vitro. It was eventually isolated, in 2000....*") and Bentley (see page 637 column 2: "*Isolation of the bacterium Tropheryma Whipplei was achieved in 2000, in a long term culture system with human fibroblasts, with a reported generation time of 18 days*" (Raoult et al 2000)).

14. I hereby declare that all statements made herein of my own knowledge are true, and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine and/or imprisonment under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing therefrom.

Date: August 18th 2005

M. Drancourt

Attachment:
December 22, 1998 Schoedon letter

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Dear Prof. Drancourt

We a very sorry to say that the isolate of *T. whippelii* is no longer available. Funding of our interdisciplinary research project on that topic was abandoned based upon the opinions of a board of international reviewers, claiming Whipple's disease beeing very rare in Switzerland and especially supply of material from additional patients would be very limited. Therefore we stopped culturing *Tropheryma* and turned to our original funded projects. We regret not being able to supply you with the requested material.

Sincerely yours

J. Schoedon

PD Dr Gabriele Schoedon